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Pseudosperma flavorimosum sp. nov. from Pakistan

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Abstract—*Pseudosperma flavorimosum* is a new species described from Khyber Pakhtunkhwa province, Pakistan. It is delimited based on morphological characters combined with a molecular phylogeny inferred from nuclear ribosomal DNA internal transcribed spacer (ITS) sequence analyses. The ITS-based phylogeny supports the independence of the new species, which is also is morphologically distinct from closely related taxa.

Key words—*Agaricales*, *Basidiomycota*, *Inocybaceae*, taxonomy

Introduction

Inocybaceae Jülich (*Basidiomycota*, *Agaricales*) is one of the larger families of agaric fungi with more than 700 species distributed worldwide (Matheny & al. 2009; Kropp & al. 2010; Bougher & Matheny 2011; Bougher & al. 2012; Fan & Bau 2013, 2014; Braaten & al. 2014; Esteve-Raventós & al. 2015; Jabeen & al. 2016; Farooqi & al. 2017; Matheny & Bougher 2017; Latha & Manimohan 2017; Ullah & al. 2018; Matheny & al. 2019). The number of species has increased considerably with the exploration of tropical and southern temperate areas. Many representatives of genera of *Inocybaceae* have been reported from Asia (Kobayashi & Onishi 2010; Fan & Bau 2013, 2014; Horak & al. 2015; Latha & Manimohan 2015, 2016, 2017; Saba & al. 2015; Jabeen & al. 2016; Pradeep & al. 2016; Farooqi & al. 2017; Naseer &

al. 2017; Liu & al. 2018; Ullah & al. 2018), with 24 species reported from Pakistan (Ahmad & al. 1997, Ilyas & al. 2013, Saba & al. 2015, Jabeen & al. 2016, Farooqi & al. 2017, Naseer & al. 2017, Liu & al. 2018, Ullah & al. 2018). All these species were previously placed in *Inocybe* (Fr.) Fr., but the recent classification by Matheny & al. (2019) distributes these taxa among separate genera.

Infrageneric classifications of *Inocybe* have been based on the morphology of the basidiospores, cystidia, and stipe (Kuyper 1986), with various morphological classifications proposed by different workers. Traditionally, *I.* sect. *Rimosae* was placed in *I.* subg. *Inosperma* (Kühner 1980, Kuyper 1986, Stangl 1989, Larsson & al. 2009). Larsson & al. (2009), who studied the phylogeny within *I.* sect. *Rimosae* using multigene DNA sequence analyses, found that *I.* sect. *Rimosae* comprised two strongly supported clades: /maculata and *I.* sect. *Rimosae* s. str. (sensu Larsson & al. 2009) The species in /maculata clustered with those in *I.* sect. *Cervicolores* and these two groups collectively represent *I.* subg. *Inosperma* s. str. *Inocybe* sect. *Rimosae* s. str. [corresponding with /pseudosperma of Matheny (2005)] has emerged as an independent clade, well distinguished from *I.* subg. *Inosperma* s. str. species of *I.* sect. *Rimosae* s. str. are characterized by the lack of metuloids and pleurocystidia and the presence of cylindrical to clavate cheilocystidia; smooth, radially appressed-fibrillose to rimose pilei; and smooth, elliptical to indistinctly phaseoliform basidiospores. In their recent revised classification, Matheny & al. (2019) recognize *I.* sect. *Rimosae* s. str. as an independent genus, *Pseudosperma* Matheny & Esteve-Rav., which we recognize here.

During 2012–2014 we wished to analyze fungal communities in coniferous forests of Khyber Pakhtunkhwa, Pakistan, that host a highly diverse mycota including mycorrhizal and non-mycorrhizal species. Here we identify one species in *Inocybaceae* based on morphological and molecular analyses and reveal the phylogenetic relationships among taxa.

Materials & methods

Morphological analysis

Basidiomata were collected from two administrative divisions of Pakistan. The first sampling site—Khanian, District Mansehra, Hazara Division, Khyber Pakhtunkhwa province—lies immediately south of the main Himalayan range with typical moist temperate climate and is dominated by *Cedrus deodara* along

with *Abies pindrow* (Royle ex D. Don) Royle, and *Pinus wallichiana*. (Siddiqui & al. 2013). The second site is Mashkun, a high mountainous region in the Swat district of Malakand division, Khyber Pakhtunkhwa province. The climate is dry temperate, and forests are dominated by *C. deodara* and *P. wallichiana*, with some *A. pindrow* also present (Champion & al. 1965).

Basidiomata were collected and photographed in their natural habitat. Morphological characters were recorded from fresh specimens. Color codes follow Munsell (1975). Each collection was preserved using hot air dryers. Tissues from dried basidiomata were rehydrated in 5% KOH prior to anatomical observation under a Meiji MX4300h light microscope. Crush mounts were prepared and stained with Congo red. The abbreviation (n/m/p) represents n number of basidiospores measured from m number of fruit bodies and p number of collections. Basidiospores were measured in lateral view. The dimensions were recorded as (a–)b–c(–d), where (a) = extreme minimum value, range b–c contains minimum of 90% of the calculated values and (d) = extreme maximum value, Q indicates l/w ratio of the spores and avQ = average Q of all spores. Other hyphal measurements are given as ranges. The examined specimens are deposited in the herbarium (LAH), Department of Botany, University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan.

Molecular analysis

Genomic DNA was extracted and amplified according to White & al. (1990), Gardes & Bruns (1993), and Bruns (1995). The PCR products were purified and sequenced by Macrogen Inc. (Korea). The newly generated sequence was deposited in GenBank.

Sequences generated using forward and reverse primers were combined in the BioEdit software version 7.2.5 (Hall 1999) to obtain a consensus sequence. A sequence homology search was conducted using the BLAST algorithm (http://www.ncbi.nlm.nih.gov) on 25 May 2019. Complete ITS sequences that showed the maximum similarity and sequences of the putative closest relatives of our species according to the published literature were included in the final matrix (Larsson & al. 2009, Kropp & al. 2013, Latha & Manimohan 2017, Liu & al. 2018, Matheny & al. 2019) to reconstruct phylogeny. *Auritella foveata* C.K. Pradeep & Matheny (GU062740) served as outgroup following Larsson & al. (2009) and Matheny & al. (2019). Multiple sequences were aligned using the online webPRANK at EMBL-EBI (https://www.ebi.ac.uk/goldman-srv/webprank/). Maximum likelihood analysis was performed using General Time Reversible model (Nei & Kumar 2000) in MEGA version 6 (Tamura & al. 2013) at 1000 bootstrap pseudoreplicates by finding best-fit substitution model. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.9359)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 0.0000% sites).

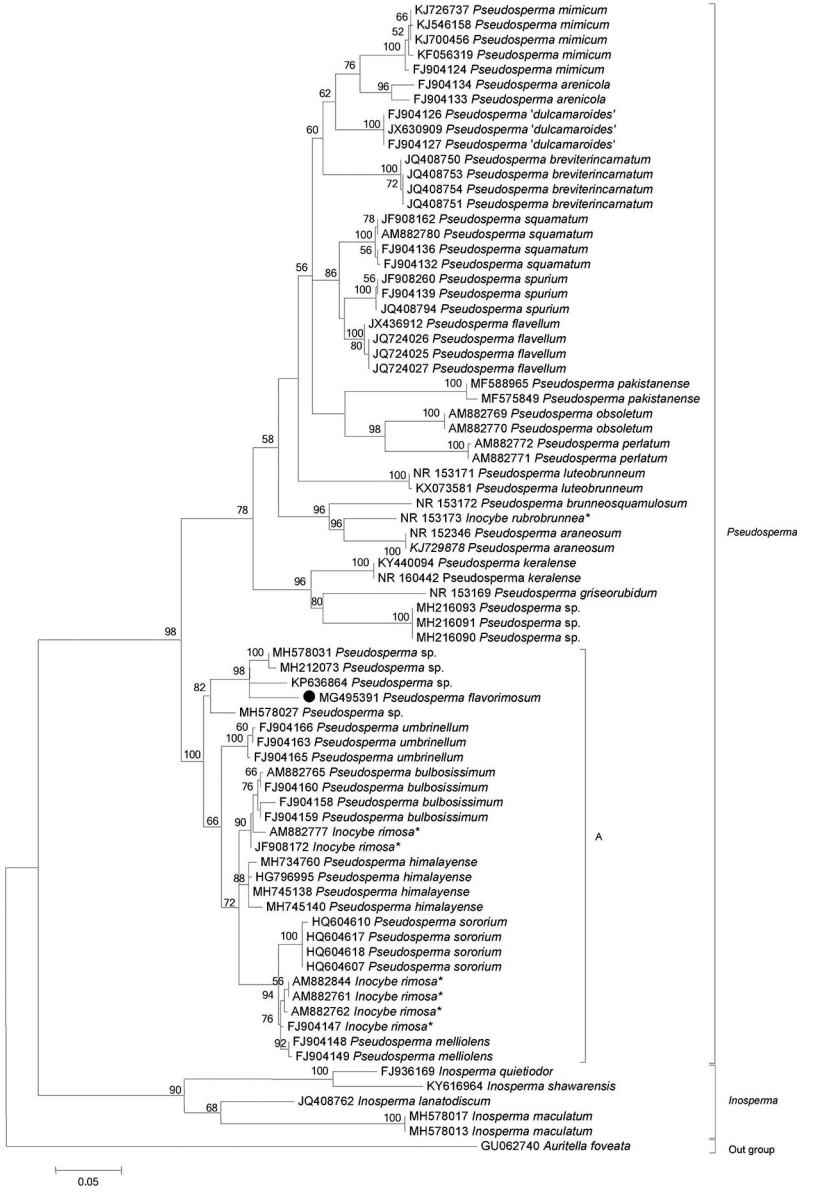

Fig. 1. Molecular phylogenetic analysis of *Pseudosperma flavorimosum* based on ITS sequences. Sequence generated during this study is marked by ●. Scale bar = nucleotide substitutions per site. The names of the taxa are written according to Matheny & al (2019). Those represented in quotes lack type specimens. * represents taxa that are not discussed in Matheny & al (2019) and given names according to their publication source and GenBank record.

Phylogeny

The NCBI BLAST search revealed that the *Pseudosperma flavorimosum* sequence (MG495391) shared 89–90% identity with unidentified "*Inocybe* sp." sequences (MH578031, MH578027, MH212073) from USA. Closely related ITS sequences were retrieved from GenBank for generating a phylogeny for the Pakistani taxon based on 77 nucleotide sequences. The final ITS matrix comprised a total of 1403 positions of which 734 were conserved, 442 were variable, 337 were parsimony informative, and 99 were singletons. The *P. flavorimosum* sequence forms a sister clade with unidentified *Inocybe* sequence KP636864, and both sequences clustered on the same branch with two more unidentified sequences (MH212073, MH578031) in the same clade as unidentified *Pseudosperma* sequence MH578027 with 82% bootstrap support and were separated from the clade containing *P. bulbosissimum* (Kühner) Matheny & Esteve-Rav., *P. himalayense* (Razaq & al.) Matheny & Esteve-Rav., *P. melliolens* (Kühner) Matheny & Esteve-Rav., *P. rimosum* (Bull.) Matheny & Esteve-Rav., *P. sororium* (Kauffman) Matheny & Esteve-Rav., and *P. umbrinellum* (Bres.) Matheny & Esteve-Rav. Our phylogenetic tree (Fig. 1) places the above *Pseudosperma* species in the same relationships as shown in Larsson & al. (2009, there as *Inocybe*) and Matheny & al. (2019).

Pseudosperma flavorimosum Jabeen & Khalid, **sp. nov.** Figs. 2, 3

MB 823494

Differs from *Pseudosperma sororium* by its smooth ellipsoidal to slightly amygdaliform basidiospores with a broad base and narrow apex.

Type: Pakistan. Khyber Pakhtunkhwa province: Hazara division, Mansehra district, Kaghan valley (Khanian), 2500 m a.s.l., on soil under *Pinus wallichiana* A.B. Jacks., 3 Aug 2014, Muhammad Burhan SJ103 (**Holotype**, LAH35042; GenBank: MG495391).

Etymology: The specific epithet (Lat.) refers to the yellowish and rimose pileus.

Pileus 45–5.1 mm wide, initially conico-convex with a prominent umbo, becoming broadly conico-convex with a prominent umbo at maturity, umbo conical when young, becoming more obtuse in maturity, surface yellowish brown (10YR8/8) with dark brown (10R4/12) fibrillose streaks, dry, fibrillose, radially rimose towards the margin, margin incurved. Lamellae adnate to adnexed, moderately close, ≤2.5 mm deep, narrow, initially yellowish brown (10YR7/8), becoming dark brown (10R6/14) at maturity, edges fimbriate. Lamellulae in different lengths. Stipe 75 × 7 mm, central, cylindrical, narrower towards the apex and wider towards the base, apex furfuraceous, base splitting, surface dry, context off white under a yellowish (10YR8/8) to dark brown (5YR2/6) cuticle.

Basidiospores [40/2/2] (7.8–)9.5–12.8(–13.31) × (5.7–)6.3–7.8(–8.9) µm, Q = (1.21–)1.39–1.91(–1.98), avQ = 1.62, elliptical, amygdaliform with broad apex and narrow base, smooth, uniguttulate. Basidia (14.8–)26.4–31.2(–36.7) × (10.0–)10.5–10.8(–11.8) µm, clavate, 4-spored, thin-walled, guttulate. Cheilocystidia (18.5–)19.2–21.7(–25.3) × (7.5–)7.7–8.6(–9.1) µm, broadly clavate, thin-walled, frequently arranged on lamellar edges. Pleurocystidia absent. Pileipellis hyphae (3.8–)4.9–6.3(–6.6) µm diam., intricate trichoderm, non-encrusted, frequently septate, filamentous, fusoid terminals, clamped septa common, hyaline. Stipitipellis hyphae (4.5–)4.7–8.7(–9.2) µm diam., trichoderm, septate, filamentous, rarely branched, clamp connections not observed.

Ecology & distribution—among conifers in Himalayan moist and dry temperate forests; known only from Pakistan.

Additional specimen examined—**PAKISTAN. Khyber Pakhtunkhwa province: Malakand division**, Swat district, Mashkun, 2500 m a.s.l., on soil under *Cedrus deodara* (Roxb. ex D. Don) G. Don, 5 Sep 2013, Sana Jabeen MTI (LAH35043).

Discussion

Pseudosperma flavorimosum is characterized by its yellowish brown pileus with dark brown striations and radially rimose margins and a stipe with a rimose base and losing its stipitipellis over time. It is characterized with smooth ellipsoidal to slightly amygdaliform basidiospores with a broad base and narrow apex and broadly clavate cheilocystidia. ITS sequence analysis clusters *P. flavorimosum* with other *Pseudosperma* species. Taxa within the genus separated into several clades, with *P. flavorimosum* clustering in subclade A. *Pseudosperma melliolens*, described from France, also looks like a typical *P. rimosum* (Bull.) Matheny & Esteve-Rav. (Bon 1997).

Pseudosperma flavorimosum shares morphological similarities with *P. sororium,* as originally described by Kauffman (1924). But the elliptical or elongate-ellipsoid basidiospores of *P. sororium* are not truly subreniform, sub-inequilateral and are obtuse at both ends, distinguishing from *P. flavorimosum*.

Pseudosperma himalayense, a recently described taxon from Pakistan, differs morphologically from *P. flavorimosum* by its paler pileus (varying from white to cream, pale yellow, olive yellow, and light brown to camel brown) with dentate margins, ellipsoid to slightly amygdaliform basidiospores, and clavate to cylindrical cheilocystidia (Liu & al. 2018).

Pseudosperma umbrinellum bears yellowish to reddish brown pileus with a dark centre and strongly rimose and lighter periphery while *P. flavorimosum*

Fig. 2. *Pseudosperma flavorimosum* (LAH35042, holotype). Basidiomata. Scale bars = 1 cm. Photos by Sana Jabeen

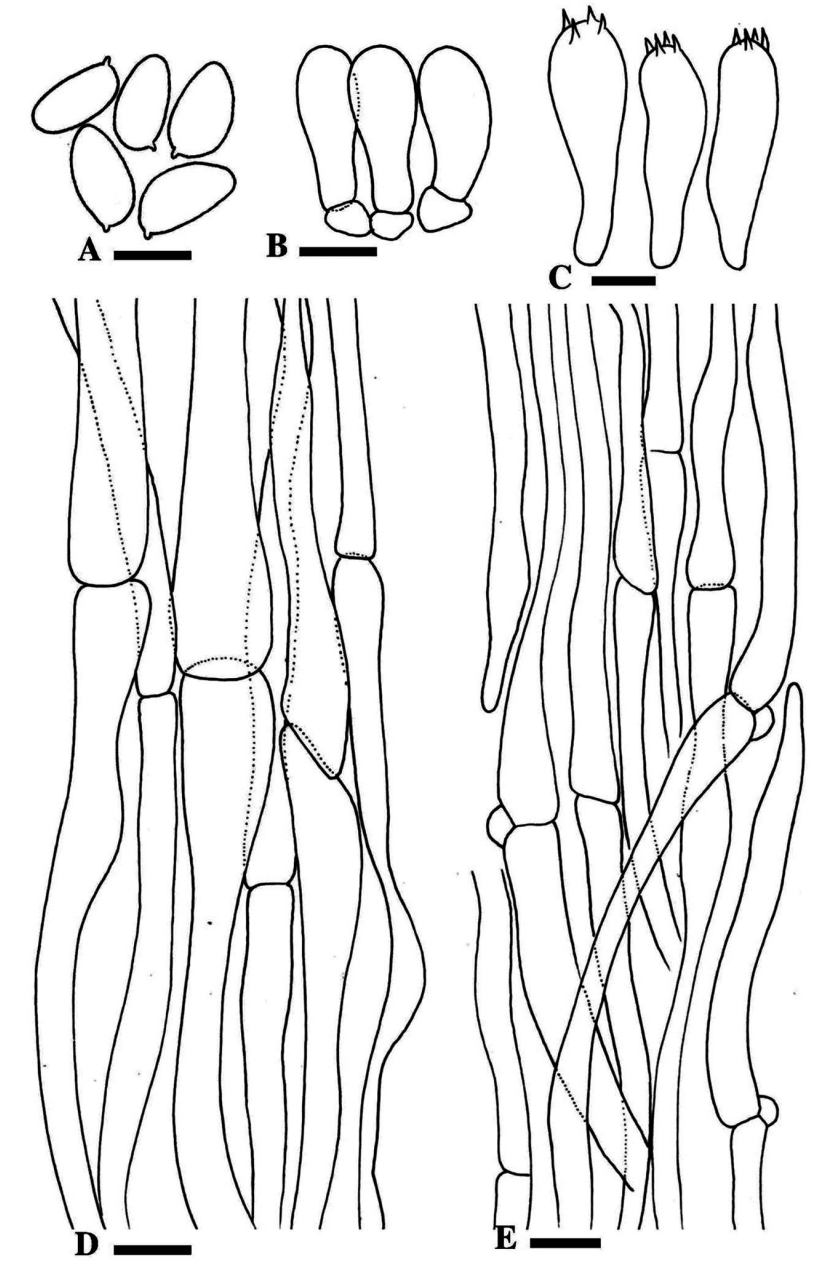

Fig. 3. *Pseudosperma flavorimosum* (LAH35042, holotype). A. Basidiospores; B. Cheilocystidia; C. Basidia; D. Stipitipellis; E. Pileipellis. Scale bars = 10 μm. Drawings by Sana Jabeen.

bears yellowish pileus with brown striations, but the anatomical features are identical to *P. rimosum*. The taxa adjacent to *P. flavorimosum* in the phylogenetic tree are undescribed morphologically but form separate lineages within subclade A of *Pseudosperma* with a strong bootstrap value supporting *P. flavorimosum* as a distinct taxon.

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